

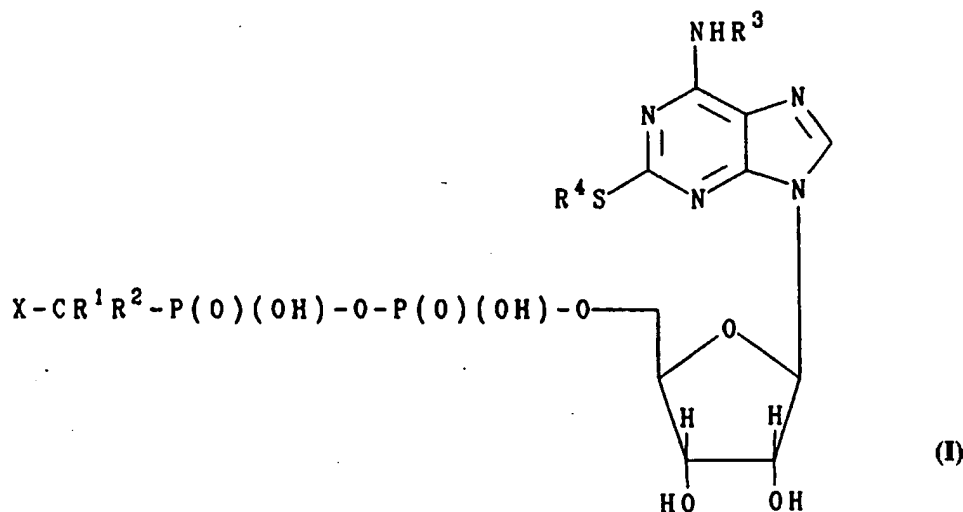
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(54) Title: N-ALKYL-2-SUBSTITUTED ATP ANALOGUES



(57) Abstract

There are disclosed compounds of formula (I) wherein R¹ and R² independently represent hydrogen or halogen, R³ and R⁴ independently represent phenyl, or alkyl C₁₋₆ optionally substituted by one or more substituents selected from OR⁵, alkylthio C₁₋₆, NR⁶R⁷, phenyl, COOR⁸ and halogen, R⁵, R⁶, R⁷ and R⁸ independently represent hydrogen or alkyl C₁₋₆, and X represents an acidic moiety, and pharmaceutically acceptable salts thereof. Processes for their production and pharmaceutical compositions and methods of treatment involving their use are also described.

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N-ALKYL-2-SUBSTITUTED ATP ANALOGUES

This invention relates to pharmaceutically useful novel compounds, processes for their production, pharmaceutical compositions containing them and methods of treatment involving their use.

Adenosine triphosphate (ATP) has potent pharmacological effects on a variety of tissues. The activity of ATP and the other extracellular adenine nucleotides, adenosine diphosphate (ADP) and adenosine monophosphate (AMP), are mediated by P_2 -purinoceptors. However, the potency of ATP in some tissues, e.g. the bladder, may be reduced due to rapid dephosphorylation, to AMP and adenosine, by ectonucleotidases present in these tissues.

In recent studies ATP analogues which are resistant to dephosphorylation have been used as biological probes to investigate the P_2 -purinoceptors present in a variety of tissues:

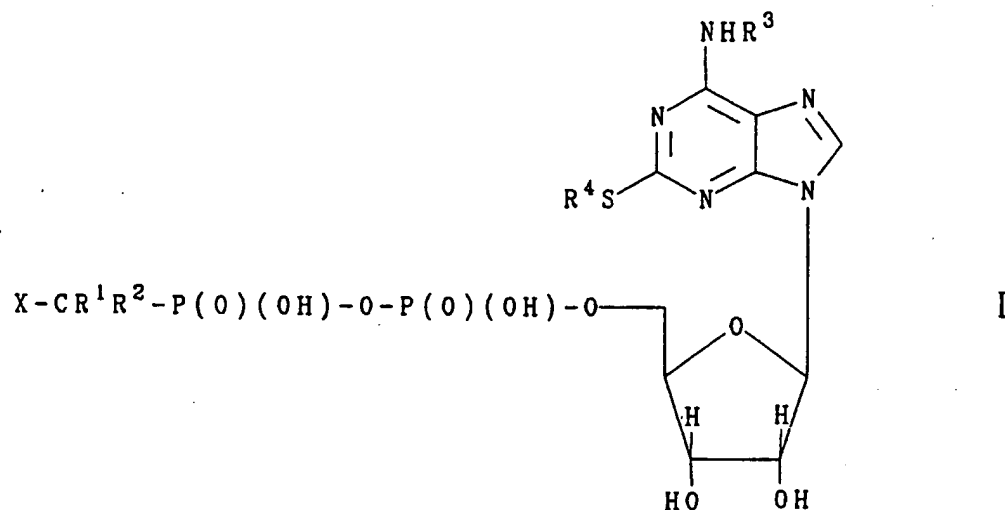
Cusack *et al*, *Br. J. Pharmacol.*, 1987, **90**, 791-795, describe the activity of 2-methylthio-5'-adenylic acid, monoanhydride with methylenebisphosphonic acid, 2-methylthio-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid and 2-methylthio-5'-adenylic acid, monoanhydride with difluoromethylenebisphosphonic acid on the guinea pig taenia coli and urinary bladder. Stone and Cusack, *Br. J. Pharmacol.*, 1989, **97**, 631-635, describe the use of *inter alia* 2-methylthio-5'-adenylic acid, monoanhydride with difluoromethylenebisphosphonic acid in an investigation of P_2 -purinoceptors in the rat hippocampus. Maguire and Satchell in "Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides", Ed. H.P. Baer and G.I. Drummond, Raven Press, New York, 1979, p.33-43, disclose the inhibition of guinea pig taenia coli by the compound 2-chloro-5'-adenylic acid, monoanhydride with methylenebisphosphonic acid.

Cusack and Hourani, *Nucleosides & Nucleotides*, 1991, **10**(5), 1019-1028, have also reported that 2-methylthio-5'-adenylic acid, monoanhydride with methylenebisphosphonic acid inhibits ADP- α -S induced platelet aggregation.

International Patent Application WO 92/17488 (Fisons plc) discloses a number of 2-substituted ATP analogues and their activity as inhibitors of platelet aggregation.

We have now found a group of novel *N*-alkyl-2-substituted ATP analogues which exhibit pharmacological activity.

According to a first aspect of the present invention, there is provided a compound of formula I,



wherein R^1 and R^2 independently represent hydrogen or halogen,

R^3 and R^4 independently represent phenyl, or alkyl C_{1-6} optionally substituted
 5 by one or more substituents selected from OR^5 , alkylthio C_{1-6} , NR^6R^7 , phenyl, $COOR^8$ and halogen,

R^5 , R^6 , R^7 and R^8 independently represent hydrogen or alkyl C_{1-6} , and

X represents an acidic moiety,

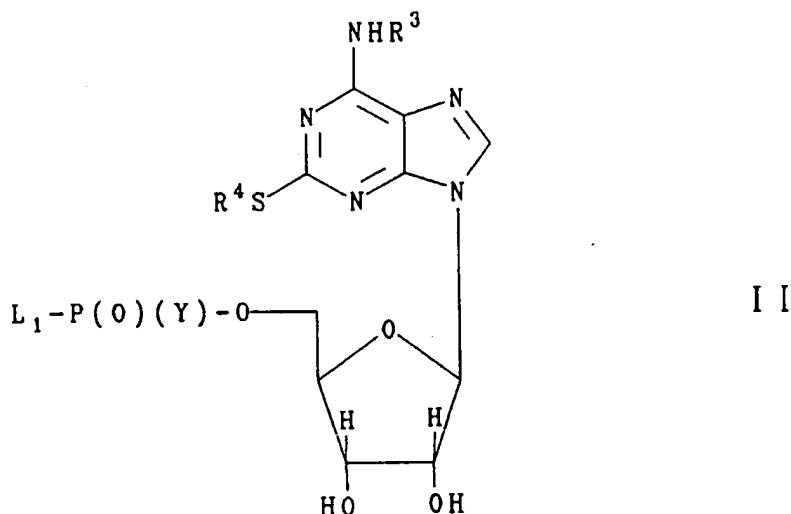
and pharmaceutically acceptable salts thereof.

10 Compounds of formula I may exist in tautomeric, enantiomeric and diastereomeric forms, all of which are included within the scope of the invention.

According to the invention there is further provided a process for the preparation of compounds of formula I, and salts thereof, which comprises:

a) reacting a compound of formula II, or a salt thereof,

3



wherein R^3 and R^4 are as defined above, L_1 represents a leaving group, and Y represents (i) OH, or (ii) a leaving group L_2 , with a compound of formula III, or a salt thereof,



wherein R^1 , R^2 and X are as defined above; followed, in the case where Y represents L_2 by hydrolysis,

b) removal of a protecting group from a corresponding protected compound of formula I in which one or more of the functional groups is protected,

and where desired or necessary converting the resultant compound of formula I, or another salt thereof, to a pharmaceutically acceptable salt thereof or vice versa.

In process a)(i), when Y represents OH, leaving groups which L_1 may represent include amines, for example, dialkylamines, or saturated or unsaturated cyclicamines; particular leaving groups which may be mentioned include morpholinyl, imidazolyl and triazolyl. The reaction is preferably carried out in a solvent, preferably a dipolar aprotic solvent, for example, pyridine, dimethylformamide, acetonitrile, hexamethylphosphorotriamide, N,N' -dimethylpropyleneurea or

1-methyl-2-pyrrolidinone. The reaction may be carried out at a temperature of from -20 to 100°C, e.g. from 10 to 30°C.

Compounds of formula II in which Y represents OH are either known or may be prepared using methods known to those skilled in the art, for example by techniques analogous to those described in International Patent Application WO 92/17488 (Fisons plc). For example, compounds of formula II in which L₁ represents morpholinyl may be prepared from the corresponding 5'-monophosphates by treatment with morpholine in the presence of a condensing agent such as dicyclohexylcarbodiimide, preferably in the presence of a protic solvent or mixture of solvents such as *t*-butanol and water.

In process a)(ii), when Y represents L₂, leaving groups that L₁ and L₂ may represent include halogen e.g. chlorine. L₁ and L₂ may be different but are preferably the same. Compounds of formula II in which Y represents L₂ may be prepared from the corresponding nucleoside by reaction with a phosphorylating agent bearing three leaving groups, i.e. POL₁L₂L₃; particular phosphorylating agents which may be mentioned include POCl₃. The resulting compound of formula II need not be isolated but may be reacted *in situ* with the compound of formula III, followed by hydrolysis, e.g. base catalysed hydrolysis using Na₂CO₃.

The nucleosides and nucleoside 5'-monophosphates used in the preparation of the compounds of formula II are either known or may be prepared from known compounds using known techniques, see, for example, "Chemistry of Nucleosides and Nucleotides" Vol. 2, Ed. Leroy B. Townsend, Plenum Press 1991.

The compounds of formula III are either known or may be prepared from known compounds using known techniques as described, for example, in International Patent Application WO 92/17488 (Fisons plc).

In the above process it may be necessary for any functional groups, e.g. hydroxy or amino groups, present in the starting materials to be protected, thus process b) may involve the removal of one or more protecting groups.

Suitable protecting groups and methods for their removal are, for example, those described in "Protective Groups in Organic Synthesis" by T. Greene and P.G.M. Wutts, John Wiley and Sons Inc., 1991. Hydroxy groups may, for example, be protected by arylmethyl groups such as phenylmethyl, diphenylmethyl or triphenylmethyl; acyl groups such as acetyl, trichloroacetyl or trifluoroacetyl; or as

tetrahydropyranyl derivatives. Suitable amino protecting groups include arylmethyl groups such as benzyl, (R,S)- α -phenylethyl, diphenylmethyl or triphenylmethyl, and acyl groups such as acetyl, trichloroacetyl or trifluoroacetyl. Conventional methods of deprotection may be used including hydrogenolysis, acid or base hydrolysis, or
5 photolysis. Arylmethyl groups may, for example, be removed by hydrogenolysis in the presence of a metal catalyst e.g. palladium on charcoal. Tetrahydropyranyl groups may be cleaved by hydrolysis under acidic conditions. Acyl groups may be removed by hydrolysis with a base such as sodium hydroxide or potassium carbonate, or a group such as trichloroacetyl may be removed by reduction with, for example,
10 zinc and acetic acid.

The compounds of formula I, and salts thereof, may be isolated from their reaction mixtures using conventional techniques.

The teachings of the documents mentioned above are herein incorporated by reference.

15 Salts of the compounds of formula I may be formed by reacting the free acid, or a salt thereof, or the free base, or a salt or derivative thereof, with one or more equivalents of the appropriate base or acid. The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, e.g. ethanol, tetrahydrofuran or diethyl ether, which may be removed *in*
20 *vacuo*, or by freeze drying. The reaction may also be a metathetical process or it may be carried out on an ion exchange resin.

Pharmaceutically acceptable salts of the compounds of formula I include alkali metal salts, e.g. sodium and potassium salts; alkaline earth metal salts, e.g. calcium and magnesium salts; salts of the Group III elements, e.g. aluminium salts;
25 and ammonium salts. Salts with suitable organic bases, for example, salts with hydroxylamine; lower alkylamines, e.g. methylamine or ethylamine; with substituted lower alkylamines, e.g. hydroxy substituted alkylamines; or with monocyclic nitrogen heterocyclic compounds, e.g. piperidine or morpholine; and salts with amino acids, e.g. with arginine, lysine etc, or an *N*-alkyl derivative thereof; or with an aminosugar,
30 e.g. *N*-methyl-D-glucamine or glucosamine. The non-toxic physiologically acceptable salts are preferred, although other salts are also useful, e.g. in isolating or purifying the product.

The compounds of formula I may exhibit tautomerism, e.g. imine-enamine tautomerism at the 6-position of adenine. The compounds also contain one or more asymmetric carbon atoms and therefore exhibit optical and/or diastereoisomerism. Diastereoisomers may be separated using conventional techniques, e.g.

5 chromatography or fractional crystallisation. The various optical isomers may be isolated by separation of a racemic or other mixture of the compounds using conventional, e.g. fractional crystallisation or HPLC, techniques. Alternatively the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation.

10 Alkyl groups which R^3 to R^8 may represent include straight, branched or cyclic, saturated or unsaturated alkyl groups.

Halogens which R^1 and R^2 may represent include F, Cl, Br and I.

We prefer compounds in which R^1 and R^2 are the same. We particularly prefer compounds in which R^1 and R^2 represent Cl.

15 We prefer compounds in which R^3 and R^4 represent alkyl C_{1-6} optionally substituted by one or more substituents selected from OR^5 , alkylthio C_{1-6} , NR^6R^7 , phenyl, $COOR^8$ and halogen.

Halogens with which R^3 and R^4 may be substituted include Cl, Br and I, and especially F.

20 We particularly prefer compounds in which R^3 represents alkyl C_{1-6} optionally substituted by alkylthio C_{1-6} . Particular alkyl groups that R^3 may represent include propyl and butyl, and especially ethyl. Particular substituted alkyl groups that R^3 may represent include 2-(methylthio)ethyl.

We particularly prefer compounds in which R^4 represents alkyl C_{1-6} optionally substituted by one or more, e.g. three, halogen atoms. Particular groups that R^4 may represent include propyl and 3,3,3-trifluoropropyl.

Acidic moieties which X may represent include Bronsted-Lowry acids, i.e. moieties which act as proton donors. The acidic moiety may be mono- or poly-acidic. Specific acidic moieties which may be mentioned include $-P(O)(OH)_2$, $-SO_3H$ and
30 $-CO_2H$.

We prefer compounds of formula I in which Z represents $-P(O)(OH)_2$.

The compounds of formula I are useful because they exhibit pharmacological activity in mammals. In particular, they show activity in the prevention of platelet aggregation.

The potency of the compounds of formula I as inhibitors of platelet aggregation may be determined from their ability to act as P_{2T} receptor antagonists, see Example X.

The compounds may be used in any condition where platelet aggregation is involved. The compounds may thus act as anti-thrombotic agents and are indicated in the treatment or prophylaxis of unstable angina, thromboembolic stroke and peripheral vascular disease. They are also indicated in the treatment or prophylaxis of the sequelae of thrombotic complications from angioplasty, thrombolysis, endarterectomy, coronary and vascular graft surgery, renal dialysis and cardio-pulmonary bypass.

Further indications include the treatment or prophylaxis of disseminated intravascular coagulation, deep vein thrombosis, pre-eclampsia/eclampsia, tissue salvage following surgical or accidental trauma, vasculitis, arteritis, thrombocythaemia, ischemia and migraine.

According to a further aspect of the invention, we therefore provide the compounds of formula I, as defined above, as pharmaceuticals.

Further, we provide the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a pharmaceutical composition for the treatment of a condition where platelet aggregation is involved.

The dosage to be administered will vary widely, depending on, amongst other factors, the particular compound of formula I employed, the mode of administration, the condition to be treated and its severity. However, in general, a total daily dose of from 0.1 mg to 1000 mg may be suitable for man, which may be administered in divided doses e.g. up to 6 times per day. If the compound is to be administered by infusion then typical a typical dosage rate for man is e.g. $0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

The compounds will generally be administered in the form of a pharmaceutical composition.

Thus, according to a further aspect of the invention there is provided a pharmaceutical composition including preferably less than 80% w/w, more preferably less than 50% w/w, e.g. 0.1 to 20%, of a compound of formula I, or a

pharmaceutically acceptable salt thereof, as defined above, in admixture with a pharmaceutically acceptable diluent or carrier.

We also provide a process for the production of such a pharmaceutical composition which comprises mixing the ingredients.

5 Examples of pharmaceutical formulations which may be used, and suitable diluents or carriers, are as follows:

for intravenous injection or infusion - purified water or saline solution;

for inhalation compositions - coarse lactose;

for tablets, capsules and dragees - microcrystalline cellulose, calcium
10 phosphate, diatomaceous earth, a sugar such as lactose, dextrose or mannitol, talc, stearic acid, starch, sodium bicarbonate and/or gelatin;

for suppositories - natural or hardened oils or waxes.

When the compound is to be used in aqueous solution, e.g. for infusion, it may be necessary to incorporate other excipients. In particular there may be
15 mentioned chelating or sequestering agents, antioxidants, tonicity adjusting agents, pH-modifying agents and buffering agents.

Solutions containing a compound of formula I may, if desired, be evaporated, e.g. by freeze drying or spray drying, to give a solid composition, which may be reconstituted prior to use.

20 When not in solution, the compound of formula I preferably is in a form having a mass median diameter of from 0.01 to 10 μm . The compositions may also contain suitable preserving, stabilising and wetting agents, solubilisers, e.g. a water-soluble cellulose polymer such as hydroxypropyl methylcellulose, or a water-soluble glycol such as propylene glycol, sweetening and colouring agents and
25 flavourings. Where appropriate, the compositions may be formulated in sustained release form.

According to a further aspect of the invention, we provide a method of treating a condition where platelet aggregation is involved which comprises administering a therapeutically effective amount of a compound of formula I, as
30 defined above, to a patient suffering from such a condition.

The compounds of the invention are advantageous in that they may produce fewer side effects e.g. have a reduced capacity to produce hypothermia as determined by the procedure of Example Y, may have advantageous duration, may

be less toxic, more efficacious, more potent, more stable, more easily absorbed, more readily cleared from the body, exhibit a broader range of activity, or have other useful pharmacological properties, when compared with compounds known from the prior art.

5 The invention is illustrated, but in no way limited, by the following Examples, in which temperatures are given in degrees Celsius. Examples are named using Chemical Abstracts nomenclature.

Example 1

10 N-Ethyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

a) N-Ethyl-2-(propylthio)adenosine

9-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)-6-chloro-2-(propylthio)purine (1.3 g) and ethylamine (1.6 ml) in dioxane (30 ml) and water (30 ml) were heated in a sealed autoclave at 110° for 20 hours. On cooling to room temperature, evaporation
15 gave a residue which was recrystallised from ethyl acetate. Further purification by chromatography (SiO₂, methanol:ethyl acetate, 1:15 as eluant) gave the sub-title compound (0.46 g).

MS (FAB): 370 (M+H, 100%), 238 (30%).

b) N-Ethyl-2-(propylthio)-5'-adenylic acid, monoammonium salt

20 Phosphorus oxychloride (0.66 g) was added to a stirred solution of the product of step a) (0.4 g) in triethyl phosphate (12 ml) at 0°. After 4½ hours the reaction mixture was poured onto ice/water (100 g) containing sodium hydrogen carbonate (1.45 g). After 45 min the solution was washed with ether (2 x 100 ml) and applied to a column of Dowex 50W X 8 (H⁺ form). The column was washed
25 with water until the eluate was at pH 6, then eluted with 2M ammonium hydroxide. Lyophilisation gave the sub-title compound (0.32 g).

³¹P NMR δ (D₂O): 2.03 (s).

c) N-Ethyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

30 The product of step b) (0.38 g) and tributylamine (0.15 g) were combined in a small volume of pyridine and the solution evaporated to dryness. Azeotropic drying with pyridine (3 x 15 ml) followed by anhydrous N,N-dimethylformamide

(DMF) (2 x 15 ml) left a residue which was taken into anhydrous DMF (10 ml). Carbonyldiimidazole (0.66 g) was added and the reaction left at room temperature for 4 hours before adding methanol (0.209 g). After 30 min dichloromethylene-bisphosphonic acid, mono(tributylammonium) salt (2.09 g) in anhydrous DMF (30 ml) was added and the mixture stirred at room temperature for 18 hours. Filtration and evaporation afforded a residue which was purified by chromatography (DEAE-Sephadex, triethylammonium bicarbonate 0M to 0.6M as eluant). Lyophilisation gave the triethylammonium salt which was converted to the sodium form by dissolution in methanol (2 ml) and addition of sodium iodide solution (1M in acetone, 30 ml). The precipitate was collected by centrifugation, washing by repeated suspension in acetone (4 x 40 ml) and recentrifugation. Finally the solid was dissolved in water and lyophilised to give the title salt as a colourless powder (0.25 g).

^{31}P NMR δ (D_2O): 9.00 (d, $J = 18.6\text{Hz}$), 1.18 (dd, $J = 18.6\text{Hz}$, $J = 30.4\text{Hz}$), -9.35 (d, $J = 30.4\text{Hz}$).

Example 2

N-Butyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

a) *N*-Butyl-2-(propylthio)adenosine

The sub-title compound was prepared according to the method of Example 1a).

MS (FAB): 398 ($\text{M} + \text{H}^+$, 100%).

b) *N*-Butyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

Phosphorus oxychloride (1.39 g) was added dropwise, with cooling, to a solution of the product of step a) (1.8 g) in triethyl phosphate (50 ml). The resulting solution was stirred at room temperature for 4 hours.

Tributylamine (2.16 ml) was added to a stirred suspension of dichloromethylenebisphosphonic acid, mono(tributylammonium) salt (5.76 g) in triethyl phosphate (60 ml). After stirring at room temperature for 1 hour the resulting solution was added over 15 min to the solution described above. Upon stirring for a further 4 hours the reaction mixture was poured onto a 5% aqueous sodium bicarbonate solution (113 ml) then stirred for 18 hours. The resulting solution was

washed with ether (4 x 50 ml) then freeze-dried. Purification (Reversed-phase C₁₈ silica, 4% saline followed by water as eluant) gave the title salt as a colourless solid (0.69 g).

³¹P NMR δ (D₂O): 9.70 (d, J = 18.4Hz), 3.4 (dd, J = 18.4Hz, J = 30.5Hz),
5 -9.19 (d, J = 30.5Hz).

Example 3

The following compounds were prepared according to the method of Example 2.

a) N-Propyl-2-(propylthio)-5'-adenylic acid, monoanhydride with
10 dichloromethylenebisphosphonic acid, tetrasodium salt

i. N-Propyl-2-(propylthio)adenosine

MS (FAB): 384 (M+H⁺, 100%).

ii. N-Propyl-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, tetrasodium salt

15 ³¹P NMR δ (D₂O): 8.92 (d, J = 18.5Hz), 1.07 (dd, J = 18.7Hz, J = 29.0Hz),
-9.4 (d, J = 29.4Hz).

b) N-(1-Methylethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, diammonium salt

i. N-(1-Methylethyl)-2-(propylthio)adenosine

20 MS (FAB): 384 (M+H⁺, 100%), 252.

ii. N-(1-Methylethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, diammonium salt

Further purification of the crude product by chromatography
(DEAE-Sephadex, ammonium bicarbonate solution, 0M to 0.6M as eluant) gave the
25 title salt.

³¹P NMR δ (D₂O): 8.71 (d, J = 18.6Hz), 0.38 (dd, J = 19.1Hz, J = 28.7Hz),
-9.49 (d, J = 29.0Hz).

c) N-(2-Methoxyethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, tetrasodium salt

30 i. N-(2-Methoxyethyl)-2-(propylthio)adenosine

MS (FAB): 400 (M+H⁺, 100%), 268.

- ii. N-(2-Methoxyethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt
 ^{31}P NMR δ (D_2O): 9.05 (d, $J = 18.7\text{Hz}$), 1.44 (dd, $J = 18.8\text{Hz}$, $J = 29.3\text{Hz}$), -9.40 (d, $J = 29.5\text{Hz}$).
- 5 d) N-Cyclopentyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt
- i. N-Cyclopentyl-2-(propylthio)adenosine
MS (FAB): 410 ($\text{M}+\text{H}^+$), 278 (100%).
- ii. N-Cyclopentyl-2-(propylthio)-5'-adenylic acid, monoanhydride with
10 dichloromethylenebisphosphonic acid, tetrasodium salt
Analysis found C:24.43; H:4.43; N:7.52; S:3.76%;
 $\text{C}_{19}\text{H}_{26}\text{Cl}_2\text{N}_5\text{Na}_4\text{O}_{12}\text{P}_3\text{S}\cdot 7\text{H}_2\text{O}$ requires C:24.56; H:4.30; N:7.53; S:3.44%.
- e) N-Phenyl-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, tetrasodium salt
- 15 i. N-Phenyl-2-(propylthio)adenosine
MS (FAB): 418 ($\text{M}+\text{H}^+$, 100%), 278.
- ii. N-Phenyl-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, tetrasodium salt
 ^{31}P NMR δ (D_2O): 8.98 (d, $J = 18.3\text{Hz}$), 2.70 (dd, $J = 18.3\text{Hz}$, $J = 30.6\text{Hz}$),
20 -9.89 (d, $J = 30.6\text{Hz}$).
- f) N-(2,2,2-Trifluoroethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, tetrasodium salt
- i. N-(2,2,2-Trifluoroethyl)-2-(propylthio)adenosine
MS (FAB): 424 ($\text{M}+\text{H}^+$), 292 (100%).
- 25 ii. N-(2,2,2-Trifluoroethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with
dichloromethylenebisphosphonic acid, tetrasodium salt
MS (FAB): 822, 820, 818 ($\text{M}+\text{H}^+$), 115 (100%).
- g) N-(Methoxycarbonylmethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride
with dichloromethylenebisphosphonic acid, triammonium salt
- 30 i. N-(Methoxycarbonylmethyl)-2-(propylthio)adenosine
Analysis found C:46.44; H:5.43; N:16.80; S:7.67%;
 $\text{C}_{16}\text{H}_{23}\text{N}_5\text{O}_6\text{S}$ requires C:46.48; H:5.61; N:16.94; S:7.76%.

ii. N-(Methoxycarbonylmethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt

Further purification of the crude product by chromatography (DEAE-Sephadex, ammonium bicarbonate solution, 0M to 0.6M as eluant) gave the
5 title salt.

^{31}P NMR δ (D_2O): 9.05 (d, $J = 18.7\text{Hz}$), 1.44 (dd, $J = 18.8\text{Hz}$, $J = 29.3\text{Hz}$), -9.40 (d, $J = 29.5\text{Hz}$).

h) N-[2-(Methylthio)ethyl]-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt

10 i. N-[2-(Methylthio)ethyl]-2-(propylthio)adenosine
MS (FAB): 416 ($\text{M}+\text{H}^+$, 100%).

ii. N-[2-(Methylthio)ethyl]-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt

Further purification of the crude product by chromatography
15 (DEAE-Sephadex, ammonium bicarbonate solution, 0M to 0.6M as eluant) gave the title salt.

^{31}P NMR δ (D_2O): 8.68 (d, $J = 18.6\text{Hz}$), 0.33 (dd, $J = 18.9\text{Hz}$, $J = 29.0\text{Hz}$), -9.53 (d, $J = 29.0\text{Hz}$).

j) N-[2-(N,N-Dimethylamino)ethyl]-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, trisodium salt

i. N-[2-(N,N-Dimethylamino)ethyl]-2-(propylthio)adenosine
MS (FAB): 413 ($\text{M}+\text{H}^+$, 100%).

ii. N-[2-(N,N-Dimethylamino)ethyl]-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, trisodium salt
25 MS (FAB): 789, 787, 785 ($\text{M}+\text{H}^+$), 93 (100%).

Example 4

2-(Cyclohexylthio)-N-ethyl-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

a) 9-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)-6-chloro-2-(cyclohexylthio)purine
30 Dicyclohexyl disulfide (51.5 g) and isoamyl nitrite (16.96 g) were added to a solution of 2-amino-9-(2,3,5-tri-O-acetyl- β -D-ribofuranosyl)-6-chloropurine (10.0 g) in acetonitrile (200 ml). The solution was degassed with nitrogen then heated at 60° for

16 hours. The solution was concentrated and the residue purified (SiO₂, ethyl acetate:petroleum ether, 1:1 as eluant) to give the sub-title compound as an orange gum (3.88 g).

MS (EI): 528, 526 (M⁺), 43 (100%).

5 b) 2-(Cyclohexylthio)-N-ethyl-adenosine

The sub-title compound was prepared according to the method of Example 1a) using the product of step a).

MS (FAB): 410 (M+H⁺, 100%).

10 c) 2-(Cyclohexylthio)-N-ethyl-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

The title salt was prepared according to the method of Example 2b) using the product of step b).

³¹P NMR δ (D₂O): 9.85 (d, J = 18.5Hz); 3.85 (dd, J = 18.5Hz, J = 30.4Hz), -9.07 (d, J = 30.4Hz).

15 Example 5

N-Ethyl-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, trisodium salt

a) 2-[(3,3,3-Trifluoropropyl)thio]adenosine

A suspension of sodium hydride (60%, 1.453 g) and adenosine-2-thione monohydrate (5.35 g) in DMF (80 ml) was stirred at room temperature for 1 hour before adding 3-chloro-1,1,1-trifluoropropane (6.6 ml). After stirring for 5 days the solution was concentrated and the residue partitioned between ethyl acetate (250 ml) and water (150 ml). The organic phase was dried then concentrated. Purification (SiO₂, dichloromethane:methanol, 9:1 as eluant) gave the sub-title compound as a colourless solid (5.55 g).

MS (FAB): 396 (M+H⁺, 100%).

b) N-Acetyl-2-[(3,3,3-trifluoropropyl)thio]adenosine-2',3',5'-triacetate

The product of step a) (5.28 g) and anhydrous sodium acetate (0.723 g) in acetic anhydride (42 ml) were stirred at 80° for 6½ hours. The solution was diluted with water (100 ml), stirred at room temperature for 18 hours then extracted with dichloromethane (4 x 200 ml). The combined organic extracts were washed with saturated sodium bicarbonate solution (200 ml) then evaporated and the residue

chromatographed (SiO₂, diethyl ether:methanol, 97:3 as eluant) to give the sub-title compound as a colourless foam (5.35 g).

MS (FAB): 564 (M+H⁺), 139 (100%).

c) N-Acetyl-N-ethyl-2-[(3,3,3-trifluoropropyl)thio]adenosine-2',3',5'-triacetate

5 The product of step b) (5.12 g) in DMF (100 ml) was added over 3 hours to a suspension of sodium hydride (60%, 0.443 g) in DMF (100 ml) containing ethyl iodide (2.2 ml). After stirring for 2 days the solution was evaporated and the residue taken into ethyl acetate (300 ml) then washed with water (3 x 100 ml). The organic phase was concentrated and the residue purified (SiO₂, ethyl acetate:cyclohexane, 1:1
10 as eluant) to give the sub-title compound as a yellow gum (4.54 g).

MS (FAB): 592 (M+H⁺), 139 (100%).

d) N-Ethyl-2-[(3,3,3-trifluoropropyl)thio]adenosine

The product of step c) (4.54 g) in sodium hydroxide solution (0.1M in methanol, 155 ml) was heated at reflux for 30 min. On cooling to room temperature,
15 glacial acetic acid (0.89 ml) was added and the solution concentrated. Purification (SiO₂, dichloromethane:methanol, 95:5 as eluant) gave the sub-title compound as a colourless solid (2.73 g).

MS (FAB): 424 (M+H⁺, 100%).

e) N-Ethyl-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with
20 dichloromethylenebisphosphonic acid, trisodium salt

The title salt was prepared according to the method of Example 2b) using the product of step d).

³¹P NMR δ (D₂O): 8.89 (d, J = 18.0Hz), 2.34 (dd, J = 18.0Hz, J = 30.0Hz), -9.90 (d, J = 30.0Hz).

25 Example 6

The following compounds were prepared according to the method of Example 2:

a) N-(2,2,2-Trifluoroethyl)-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt

30 i. N-Acetyl-N-(2,2,2-trifluoroethyl)-2-[(3,3,3-trifluoropropyl)thio]adenosine-2',3',5'-triacetate

MS (FAB): 646 (M+H⁺).

- ii. N-(2,2,2-Trifluoroethyl)-2-[(3,3,3-trifluoropropyl)thio]adenosine
MS (FAB): 478 (M+H⁺), 346 (100%).
- iii. N-(2,2,2-Trifluoroethyl)-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt
5 ³¹P NMR δ (D₂O): 8.82 (d, J = 18.6Hz), 0.63 (dd, J = 18.9Hz, J = 28.9Hz), -9.43 (d, J = 29.0Hz).
- b) N-[2-(Methylthio)ethyl]-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt
- i. N-Acetyl-N-[2-(Methylthio)ethyl]-2-[(3,3,3-trifluoropropyl)thio]-
10 adenosine-2',3',5'-triacetate
MS (FAB): 638 (M+H⁺), 139 (100%).
- ii. N-[2-(Methylthio)ethyl]-2-[(3,3,3-trifluoropropyl)thio]adenosine
Analysis found C:40.70; H:4.82; N:14.79; S:13.60%;
C₁₆H₂₂F₃N₅O₄S₂ requires C:40.90; H:4.72; N:14.92; S:13.70%.
- 15 iii. N-[2-(Methylthio)ethyl]-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, triammonium salt
Further purification of the crude product by chromatography (DEAE-Sephadex, ammonium bicarbonate solution, 0M to 0.6M as eluant) gave the title salt.
- 20 ³¹P NMR δ (D₂O): 8.77 (d, J = 18.7Hz), 0.38 (dd, J = 18.9Hz, J = 27.4Hz), -9.43 (d, J = 28.8Hz).

Example 7

N-(2-Methoxyethyl)-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

- 25 a) N-(2-Methoxyethyl)-2-[(3,3,3-trifluoropropyl)thio]adenosine

A solution of the compound of Example 5b) (4.8 g), 2-bromoethyl methyl ether (1.2 ml) and potassium carbonate (1.77 g) in dry DMF (190 ml) was stirred at room temperature for 3 days. Further quantities of 2-bromoethyl methyl ether (1.2 ml) and potassium carbonate (1.77 g) were added and the mixture stirred at 40° for
30 24 hours. The reaction mixture was filtered and the filtrate concentrated to give an oil which was partitioned between ethyl acetate (200 ml) and water (200 ml). The organic phase was dried then concentrated. The resulting gum was dissolved in a

0.1M solution of sodium methoxide in methanol (180 ml) then heated at reflux for 45 min. After neutralising with acetic acid the solution was concentrated and the residue purified (SiO_2 , dichloromethane:methanol, 92:8 as eluant) to give the sub-title compound as a colourless solid (3.41 g).

MS (FAB): 454 ($\text{M}+\text{H}^+$, 100%).

b) N-(2-Methoxyethyl)-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid, tetrasodium salt

The title salt was prepared according to the method of Example 2b) using the compound of step a).

^{31}P NMR δ (D_2O): 9.88 (d, $J = 19.0\text{Hz}$), 3.80 (dd, $J = 19.0\text{Hz}$, $J = 31.0\text{Hz}$), -9.12 (d, $J = 31.0\text{Hz}$).

Example X

Quantification of P_{77} receptor agonist/antagonist activity in washed human platelets.

Preparation

Human venous blood (100 ml) was divided equally between 3 tubes, each containing 3.2% trisodium citrate (4 ml) as anti-coagulant. The tubes were centrifuged for 15 min at 240G to obtain a platelet-rich plasma (PRP) to which 300 ng.ml^{-1} prostacyclin (PGI_2 , 3 $\mu\text{l.ml}^{-1}$ PRP of 1/10 diln. in saline from stock 1 mg.ml^{-1} in ethanol) was added to stabilize the platelets during the washing procedure. Red cell free PRP was obtained by centrifugation for 10 min at 125G followed by further centrifugation for 15 min at 640G. The supernatant was discarded and the platelet pellet resuspended in modified, calcium free, Tyrode solution [(10 ml) CFT, composition: NaCl 137mM (8 g.l^{-1}), NaHCO_3 11.9mM (1 g.l^{-1}), NaH_2PO_4 0.38mM (0.06 g.l^{-1}), KCl 2.86mM (1 ml of 20% soln. l^{-1}), MgCl_2 1.05mM (1 ml of 10% soln. l^{-1}), dextrose 5.55mM (1 g.l^{-1})], gassed with 95% O_2 /5% CO_2 and maintained at 37°. Following addition of a further 300 ng.ml^{-1} PGI_2 , the pooled suspension was centrifuged once more for 15 min at 640G. The supernatant was discarded and the platelets resuspended initially in 10 ml CFT with further CFT added to adjust the final platelet count to $2 \times 10^5 \mu\text{l}^{-1}$. This final suspension was stored in a 60 ml syringe at 3° with air excluded.

To allow recovery from PGI_2 -inhibition of normal function, platelets were used in aggregation studies no sooner than 2 hours after final resuspension. In all

studies 430 μ l aliquots of platelet suspension were added to siliconized aggregation ,
cuvettes containing CaCl_2 solution (10 μ l of 45mM solution, final concentration
1mM) and stirred at 900 rpm in a PAP4 aggregometer (Biodata). Human fibrinogen
(Sigma, F 4883) and 8-sulphophenyltheophylline (8-SPT, to block any P_1 agonist
5 activity of compounds) were added to give final concentrations of 0.2 mg/ml (10 μ l of
10 mg.ml⁻¹ solution of clottable protein in saline) and 3×10^{-4} M (10 μ l of 5.6 mg.ml⁻¹
solution in 6% glucose), respectively. Recording of aggregation was then started.

Protocol

a) Selection of submaximal ADP concentration

10 A concentration of ADP producing a just submaximal response was selected
by constructing a concentration/response curve over the range 10-300 μ M. The
appropriate solution of ADP was added to the aggregation cuvette in a volume of
10 μ l, 20 min after starting the aggregation trace. Aggregation responses were
measured using the maximum rate of change in light transmission, an index given by
15 the PAP4 slope-reader. The submaximal concentration of ADP selected at this stage
of the protocol was used in the subsequent assessment of antagonist potency of the
compounds. All measurements were made in duplicate in platelets from each donor.

b) Assessment of agonist/antagonist potency

5 min after starting the aggregation trace, saline or the appropriate solution
20 of test compound was added to an aggregation cuvette in a volume of 30 μ l to give a
final concentration of 0, 10, 100 or 1000 μ M. Aggregation at this point was indicative
of agonist activity and, if it occurred, agonist potency was estimated by comparison
with control ADP responses obtained in a).

If aggregation did not occur the previously selected submaximal
25 concentration of ADP was added in a volume of 10 μ l, 15 min after the test
compound. Antagonist potency was estimated as a % inhibition of the control ADP
response to obtain an approximate IC_{50} . Compounds which completely inhibited the
ADP response at the initial concentrations were retested at a lower concentration
range. Compounds with an $\text{IC}_{50} < 10^{-8}$ M were also retested in the absence of 8-SPT
30 to confirm the lack of any P_1 agonist activity and with a 2 min rather than a 15 min
incubation to check whether inhibition was time dependent.

Results

Representative data obtained for the compounds of formula I are reported as the negative logarithm of the antagonist potency (pIC_{50}) in Table 1.

Example No.	pIC_{50}
1	9.04 ± 0.15
5	8.68 ± 0.33
6a	9.14 ± 0.11

TABLE 1

Example Y

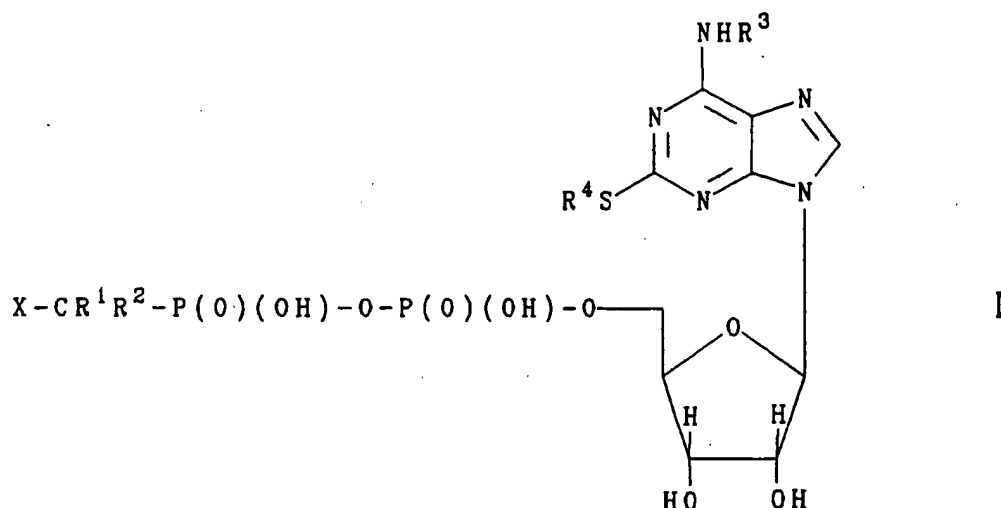
10 Measurement of Hypothermic Activity in Conscious Mice

CR/CD-1 mice (25-45 g) were used. The mice were weighed and their initial rectal temperature measured using a thermistor probe. Each animal was placed in a restraining cone and the tail vein cannulated using a 27G hypodermic needle connected to a polythene cannula and taped in position. The animal was treated
15 with a dose of the test compound administered as a 10 min intravenous infusion at a rate of $0.5 \text{ ml.kg}^{-1}.\text{min}^{-1}$. Rectal temperature was measured $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4, 6 and 24 hours after termination of the infusion.

The mean maximum reduction in rectal temperature was plotted against dose for the compound and the dose required to lower temperature by 5° was
20 determined.

CLAIMS

1. A compound of formula I,



- wherein R^1 and R^2 independently represent hydrogen or halogen,
 R^3 and R^4 independently represent phenyl, or alkyl C_{1-6} optionally substituted
 5 by one or more substituents selected from OR^5 , alkylthio C_{1-6} , NR^6R^7 , phenyl, $COOR^8$ and halogen,
 R^5 , R^6 , R^7 and R^8 independently represent hydrogen or alkyl C_{1-6} , and
 X represents an acidic moiety,
 and pharmaceutically acceptable salts thereof.
- 10 2. A compound of formula I, as claimed in Claim 1, or a pharmaceutically acceptable salt thereof, wherein X is $-P(O)(OH)_2$.
3. A compound of formula I, as claimed in Claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein R^4 is alkyl C_{1-6} optionally substituted by halogen.
4. A compound of formula I, as claimed in any one of the preceding claims, or
 15 a pharmaceutically acceptable salt thereof, wherein R^1 and R^2 are Cl.
5. A compound of formula I, as claimed in any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein R^3 is alkyl C_{1-6} optionally substituted by alkylthio C_{1-6} .
6. A compound of formula I, as claimed in Claim 1, which is
 20 *N*-Ethyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-Ethyl-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-[2-(Methylthio)ethyl]-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

5 or a pharmaceutically acceptable salt of any one thereof.

7. A compound of formula I, as claimed in Claim 1, which is

N-Butyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-Propyl-2-(propylthio)-5'-adenylic acid, monoanhydride with
10 dichloromethylenebisphosphonic acid,

N-(1-Methylethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-(2-Methoxyethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

15 *N*-Cyclopentyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-Phenyl-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-(2,2,2-Trifluoroethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with
20 dichloromethylenebisphosphonic acid,

N-(Methoxycarbonylmethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-(2-Methylthioethyl)-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

25 *N*-[2-(*N,N*-Dimethylamino)ethyl]-2-(propylthio)-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

2-(Cyclohexylthio)-*N*-ethyl-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

N-(2,2,2-Trifluoroethyl)-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid,
30 monoanhydride with dichloromethylenebisphosphonic acid,

N-(2-Methoxyethyl)-2-[(3,3,3-trifluoropropyl)thio]-5'-adenylic acid, monoanhydride with dichloromethylenebisphosphonic acid,

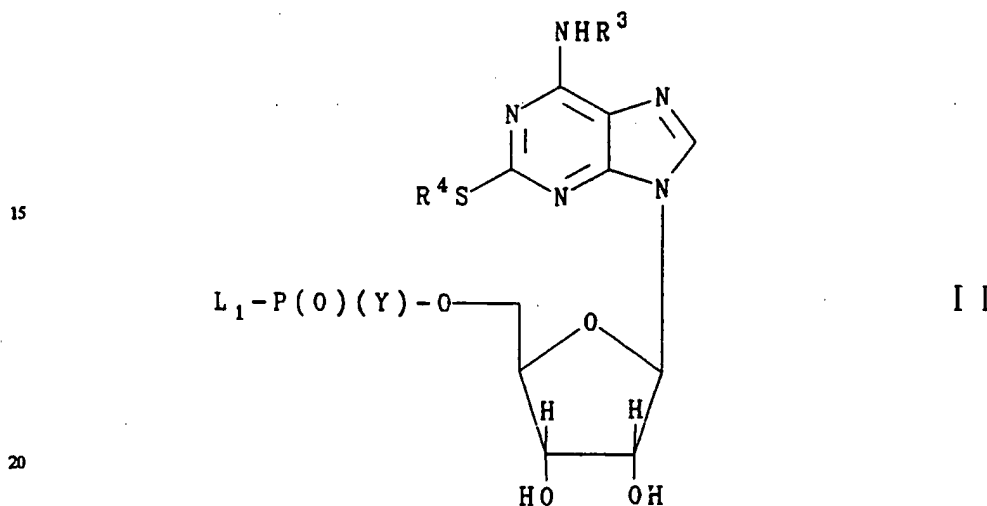
or a pharmaceutically acceptable salt of any one thereof.

8. A pharmaceutical composition including a compound of formula I, as defined in any of the preceding claims, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable carrier or diluent.

9. The use of a compound of formula I, as defined in any one of Claims 1 to 7, or a pharmaceutically acceptable salt thereof, in the manufacture of a pharmaceutical composition for the treatment of a condition where platelet aggregation is involved.

10. A process for the preparation of a compound of formula I as defined in any one of Claims 1 to 7, or a pharmaceutically acceptable salt thereof, which comprises:

a) reacting a compound of formula II, or a salt thereof,



wherein R³ and R⁴ are as defined above, L₁ represents a leaving group, and Y represents (i) OH, or (ii) a leaving group L₂, with a compound of formula III, or a salt thereof,



wherein R¹, R² and X are as defined above; followed, in the case where Y represents L₂, by hydrolysis.

b) removal of a protecting group from a corresponding protected compound of formula I in which one or more of the functional groups is protected;

and where desired or necessary converting the resultant compound of formula I, or another salt thereof, to a pharmaceutically acceptable salt thereof or vice versa.

INTERNATIONAL SEARCH REPORT

1

International application No.

PCT/GB 94/00237

A. CLASSIFICATION OF SUBJECT MATTER

IPC : C07H 19/20, A61K 31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : C07H, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9217488 (FISONS PLC), 15 October 1992 (15.10.92)	1-10
A	STN International, File CA, Chemical Abstracts, volume 115, no. 17, 28 July 1991 (Columbus Ohio, US), N J Cusack et al: "Design, syntheses and pharmacology of ATP analogs selective for subtypes of P2-purinoceptors", abstract no. 173931a, & Nucleosides Nucleotides, 10(5), 1019-28, 1991	1-10

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

3 May 1994

Date of mailing of the international search report

18.05.94

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 94/00237

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	STN International, File CA, Chemical Abstracts, volume 108, no. 21, 23 May 1988, (Columbus Ohio, US), P E R Tatham et al: "Characterization of the ATP4- receptor that mediates permeabilization of rat mast cells", abstract no. 182489v, & Eur. J. Pharmacol., 147(1), 13-21, 1988 --	1-10
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INTERNATIONAL SEARCH REPORT
Information on patent family members

SI/06

16/04/94

International application No.

PCT/GB 94/00237

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		CA-A- 2107667	07/10/92
		CN-A- 1068574	03/02/93
		EP-A- 0508687	14/10/92
		EP-A- 0579643	26/01/94
		HU-D- 9302812	00/00/00
